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Capsugel Division of Pfizer Inc.
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Greenwood, SC 29646

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August 26, 2003

National Organic Standards Board
C/o Robert Pooler, Agriculture Marketing Specialist
USDA/AMS/TM/NOP, Room 2510-So.
Ag Stop 0268, P.O. Box 96456
Washington. D.C. 20090-6456

PETITION FOR LISTING OF NPCAPS™ ON THE USDA NATIONAL LIST OF ALLOWED AND PROHIBITED SUBSTANCES

The Organic Foods Production Act of 1990, as amended, established a National List of Allowed and Prohibited Substances National List) which identifies the synthetic substances that may be used, and the no synthetic substances that cannot be used, in organic production and handling operations. The Act also provides a mechanism to petition the National
Organic Standards Board to evaluate a substance for inclusion on or removal from the National List. With this petition, Capsugel request review of the empty pullulan capsules (NPCaps™) for consideration and, if appropriate, listing on the Proposed National List of Organic substances for inclusion on:

- Nonagricultural (organic) substances allowed in or on processed products labeled as "organic" or "made with organic (specified ingredients)."

NPcaps™ is a capsule manufactured by Capsugel that is intended for use in pharmaceuticals or dietary supplements. The capsule is composed of Pullulan, Carrageenan, and Potassium Chloride).

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Capsugel appreciate the time and effort that Department of Agriculture, Agricultural Marketing Services, invests in the review of petitions for organic status. Please feel free

To contact me at my phone number or e-mail address below if you have any questions on aspect of this petition.

Respectfully yours,

Jackie L. Massey

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Petition for the Inclusion of NPCaps™ on the National Organic Standards Board List of Approved Organic Substances

Based on relevant regulatory requirements, the components and composite of NPcaps™ are qualitatively acceptable for use in pharmaceuticals and dietary supplements in Japan, the US, Canada, Australia, and the EU. Furthermore, no safety issues were identified in authoritative reviews of the individual components that would raise a concern for the use of NPcaps™. However, pharmaceutical and dietary supplement products using NPcaps™ should be assessed within the context of the final pharmaceutical/dietary supplement formulation [including the active ingredient(s)] and anticipated exposure levels.

Item A:

With this petition, Capsugel Division of Pfizer is requesting the evaluation of NPCaps™ for inclusion on:

- Nonagricultural (nonorganic) substances allowed in or on processed products labeled as "organic or "made with organic (specified ingredients)."

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Item B:

NPcaps™

Pullulan

Synonyms

Pururan

CAS Registry Number

9057-02-7

Functional Category

Multiple technical effects as a food additive.

Regulatory Citations

Pullulan (No. 373) is an existing food additive permitted for general food use (included in the list of food additives from natural origin compiled and published by the Ministry of Health and Welfare on April 16, 1996). (Japan)

Pullulan, an extra cellular polysaccharide excreted by the fungus *Aureobasidium pullulans*, is generally recognized as safe (GRAS), through scientific procedures, for use in food in general, for multiple technical effects. (US)

Identified substance in cosmetics and personal care products regulated under the Food and Drugs Act that was in commerce between January 1, 1987 and September 13, 2001. (Canada)

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Carrageenan

Synonyms
Carrageenan gum; chondrus; Irish moss gelose (from *Chondrus* spp.); Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*)

CAS Registry Number
9000-07-1

Functional Category
Emulsifying, gelling, stabilizing and thickening agent. Pharmaceutic aid as a suspending agent and viscosity-increasing agent.

Regulatory Citations
Listed in United States Pharmacopeia (USP) (24th Edition).

Carrageenan (No. 95) is an existing food additive permitted for general food use (included in the list of food additives from natural origin compiled and published by the Ministry of Health and Welfare on April 16, 1996). (Japan)

Food additive permitted for direct addition to food for human consumption, in accordance with the prescribed conditions under 21 CFR 172.620. (US)
Food additive that may be used as an emulsifying, gelling, stabilizing and thickening agent, in accordance with the prescribed conditions under Item No C.15 of Table IV in Division B.16.100 of the *Food and Drugs Act and Regulations.* (Canada)

Food additive (INS Number 407) permitted for use in foodstuff, in accordance with Good Manufacturing Practice (GMP) and the prescribed conditions under

Standard 1.3.1, Schedule 1 and Schedule 2 of the *Australia New Zealand Food Standards Code.* (Australia)

Food additive (E 407) generally permitted for use in foodstuffs, in accordance with the prescribed conditions under Annex I of the *European Parliament and Council Directive No 95/2/EC*. Recently, the European Commission's Scientific Committee on Food (SCF) released its opinion on the safety of carrageenan in response to work published by Tobacman in October 2001, which questioned the safety of degraded carrageenan (poligeenan). The SCF concluded that the specification for carrageenan should be tightened to ensure than any degraded carrageenan is kept to a minimum (not more than 5 per cent below 50 kDa). (EU)

2.3.1.3 Potassium Chloride

Synonyms

Chloropotassuril; Diffu-K; Enseal; Kaleorid; Kalitabs; Kalium-Duriles; Kaon-Cl; Kaskay; Kayback; Kay-Cee-L; K-Contin; Klor-Con; K-Norm; K-Tab; Lento-Kalium; Micro-K; Nu-K; Peter-Kal; PfiKlor; Rekawan; Repone K; Slow-K; Span-K.

CAS Registry Number

7447-40-7

Functional Category

Emulsifying, gelling, stabilizing, and thickening agent. Used when the action of potassium cation is desired.

Regulatory Citations

Listed in Japanese Pharmacopoeia (14th Edition).
Listed in United States Pharmacopeia (USP) (24th Edition).
Listed in European Pharmacopoeia (3rd Edition).
Potassium chloride (No. 49) is a designated food additive (effective August 1, 2002), appearing in Table 2, pursuant to Article 3 of the Enforcement Regulations under the Japanese Food Sanitation Law. (Japan)

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Direct food substance affirmed as generally recognized as safe (GRAS) under 21 CFR 184.1622. (US)

Included in FDA Inactive Ingredient Database for use in approved drug products.

Food additive that may be used as an emulsifying, gelling, stabilizing and thickening agent, in accordance with the prescribed conditions under Item No P.8 of Table IV in Division B.16.100 of the *Food and Drugs Act and Regulations*. (Canada)

Listed on Non-Medicinal Ingredients Nomenclature List. (Canada).

Food additive (INS Number 508) permitted for use in foodstuff, in accordance with Good Manufacturing Practice (GMP) and the prescribed conditions under Standard 1.3.1, Schedule 1 and Schedule 2 of the *Australia New Zealand Food Standards Code*. (Australia)

Food additive (E 508) generally permitted for use in foodstuffs, in accordance with the prescribed conditions under Annex I of the *European Parliament and Council Directive No 95/2/EC*. (EU)

SAFETY INFORMATION

NPcaps™

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NPcaps™ capsules are composed of pullulan, carrageenan, and potassium chloride. Available metabolism and human, and nonclinical safety data were reviewed for these ingredients. The available safety information supports the safe use of these ingredients for use in capsules used in pharmaceutical and dietary supplement products. However, pharmaceutical and dietary supplement products using NPcaps™ should be assessed within the context of the final pharmaceutical/dietary supplement formulation [including the active ingredient(s)] and anticipated exposure levels.

Pullulan

Pullulan is a modified starch produced by a naturally occurring yeast, *Aureobasidium pullulans*. The basic unit of pullulan consists of a series of three alpha-1,4 linked glucose molecules repeatedly polymerized *via* alpha-1,6 linkages on the terminal glucose. Moreover, pullulan and the associated yeast have a substantial history of use in foods in Japan, and pullulan is structurally and metabolically similar to starch.

Metabolism Studies

In vivo and *in vitro* metabolism and digestion studies in rats and humans demonstrated that pullulan is minimally hydrolyzed by salivary amylase and pancreatic amylase without glucose formation (Oku *et al.*, 1979; Okada *et al.*, 1990; Yoneyama *et al.*, 1990). Enzymes of the small intestine also hydrolyze pullulan producing minimal amounts of glucose. The majority of the administered pullulan is fermented in the large intestine to short-chain fatty acids (SCFAs). Thus, the digestion and metabolic fate of pullulan is similar to that of normal starches.

Human Safety Data

Pullulan has been used in Japan, in various forms, for more than 20 years without reported adverse effects. In addition, pullulan intakes of 10 g/day were well tolerated by human volunteers taking part in a 14-day metabolism study (Yoneyama *et al.*, 1990). The only complaint, which was reported by a few of the participants, was post-intake abdominal fullness. There were no significant changes in the blood biochemistry parameters of the volunteers fed pullulan.

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Nonclinical Safety Data
In an oral lethality study, pullulan administered at doses of up to approximately 15 g/kg body weight did not cause any mortalities in mice (Kimoto *et al.*, 1997). The yeast from which pullulan is obtained, *Aureobasidium pullulans*, also was demonstrated to be relatively innocuous, as indicated by oral LD_{50} values of >24 and >40 g/kg body weight in male dd mice, and male and female Sprague-Dawley rats, respectively (Kimoto *et al.*, 1997).
Results of longer term repeated dose studies also demonstrated pullulan to be of low oral toxicity (Oku *et al.,* 1979; Kimoto *et al.,* 1997). No toxicologically significant effects were observed in rats fed diets containing as much as 40% pullulan for 9 weeks. Observations of decreased body weight and digestive tract hypertrophy in rats fed high pullulan diets (20 to 40%) can be attributed to the effect of replacing the normal nutrient content of food with indigestible carbohydrate (LRSO, 1975).
The NO(A)EL for pullulan, based on a 63-week dietary study in rats, was estimated to be 5,000 mg/kg body weight/day (Kimoto *et al.,* 1997). The only treatment-related change noted was an increase in cecal weight in female rats receiving 5,080 mg/kg body weight/day of pullulan (about 10% of diet). Increased cecal weight is a common physiological response to consumption of poorly digested polysaccharides (El-Harith *et al.*, 1976; Oku *et al.*, 1979; MacKenzie *et al.*, 1986; Olivier *et al.*, 1991).

Genotoxicity/Mutagenicity Data
Mutagenicity studies in strains of *Salmonella typhimurium* and *Escherichia coli,* using the plate incorporation method, demonstrated that pullulan was not mutagenic either with or without metabolic activation (Kuroda *et al.*, 1985; Kimoto *et al.*, 1997). Pullulan also was not found to be clastogenic in a chromosome aberration assay in Chinese hamster lung cells (Ishidate *et al.*, 1985).
Conclusion
No adverse effects of toxicological significance have been observed for pullulan in a variety of assays. Pullulan is structurally similar to starch and would not be expected (based on estimated consumption data) to introduce a substantial increase in the level of alpha-1,6 linked glucose, a minor constituent of normal starches, into the diet. Lastly, the safety of pullulan is supported by 20 years of

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human consumption in Japan and by the absence of adverse events in human trials at doses of 10 g pullulan/day to evaluate metabolism and digestion.

Although the above profile provides a general overview of the safety data for pullulan, the use of products containing this compound should be assessed within the context of anticipated exposure levels.

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Carrageenan

Carrageenan is a sulfated polygalactan isolated from various seaweeds of the class *Rhodophyceae* (especially *Chondrus crispus*, *Gigartina stellata*, *Eucheuma spinosum*) (JECFA, 1999). The three main types of carrageenan used commercially in the food industry are *iota*-, *kappa*- and *lambda*-carrageenan. "Native" (undegraded) carrageenan also is used in food. Carrageenan and its derived calcium, potassium, and sodium salts are used in foods, cosmetics, pharmaceuticals and other products for their ability to stabilize mixtures, emulsify ingredients, and thicken or gel solutions. Carrageenan is approved for use as a direct and indirect food additive in the United States (FDA, 2002). In 1974, the WHO set the Acceptable Daily Intake (ADI) of carrageenan at up to 75 mg/kg body weight (IARC, 1983). However, a group ADI of "not specified"[1] was designated recently by JECFA, and temporarily renewed in 1999, with the statement that the ADI does not apply to neonates and young infants less than 12 weeks of age. The quantity of carrageenan consumed by neonates and young infants would be negligible since their diets would be restricted to either breast milk or baby formula. Furthermore, JECFA requested clarification of the

[1] The JECFA designation for ADI of "not specified" applies to a substance "*of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health*" (JECFA, 2001).

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significance of the apparent promotional effects of high levels of carrageenan on colon cancer (JECFA, 1999). In 2001, carrageenan and processed *Eucheuma* seaweed were reevaluated based in part on data indicating a lack of a proliferative effect of carrageeenan at dietary concentrations of up to 1.5% (approximately, 750 mg/kg body weight/day) in rats (JECFA, 2001). The previously established group ADI of "not specified" was maintained, while the restriction, pertaining to neonates and young infants, on the ADI, was withdrawn.

Metabolism Studies

Results of excretion studies in animals indicate that native carrageenan is absorbed to a very limited extent. Carrageenan (200 to >5,000 mg/kg body weight/day) when administered orally in the diet or drinking water of rats and guinea pigs, was excreted quantitatively, with no signs of storage in the liver (Hawkins and Yaphe, 1965; Chen *et al.*, 1981). There is limited breakdown and fermentation of native carrageenan in the gut, and *iota*-carrageenan is more stable than *kappa*-carrageenan.

Human Safety Data

Carrageenan has been extracted from seaweeds for human food production since 1837. Over this extensive period of use there have been no reports of adverse effects. Co-administration of 20 g carrageenan with vitamin A increased the absorption of the vitamin (Kasper *et al.*, 1979). In a limited study, carrageenan in liquid infant formula was found not to be immunosuppressive (Sherry *et al.*, 1993).

Nonclinical Safety Data

Studies in various rodent species have indicated carrageenan to be of low oral toxicity, with LD_{50} values ranging from 2,640 to 9,150 mg/kg body weight (Food and Drug Research Labs, Inc., 1971; Weiner, 1991). Early studies showed an intravenous LD_{50} of <1 to <50 mg/kg body weight in rodents (Morard *et al.*, 1964; Duncan, 1965; Anderson and Soman, 1966). A single intraperitoneal injection of 125 mg/kg body weight in rats showed that *kappa*-carrageenan was nephrotoxic and hepatotoxic, while *lambda*- and *iota*- carrageenan were not (Anderson and Duncan, 1965). An inhalation study in rats provided a 4 hour LC_{50} of >930

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mg/m³, and the LD_{50} of a dermal application of carrageenan was >2,000 mg/kg body weight (Weiner, 1991).

Repeat-dose studies in rats fed carrageenan for up to 6 months showed little to no effects at 5% dietary levels (approximately, 2,500 mg/kg body weight/day), but some growth retardation at the 10 to 20% concentration (approximately, 5,000 to 10,000 mg/kg body weight/day) (Hawkins and Yaphe, 1965; Grasso *et al.*, 1973; Tomarelli *et al.*, 1974; Coulston *et al.*, 1976). No evidence of any adverse effects or significant changes were observed in pigs fed 0 to 500 mg carrageenan/kg body weight/day, in adult rhesus monkeys receiving 1% carrageenan in water (approximately, 540 mg/kg body weight/day), and in infant baboons receiving up to 5% in infant formula (approximately, 3,400 mg/kg body weight/day) for up to 23 weeks (Benitz *et al.*, 1973; Poulsen, 1973; McGill *et al.*, 1977).

Lifetime administration of *kappa*- or *lambda*-carrageenan in the diet had no effect in mice (up to approximately 42,000 mg/kg body weight/day) and caused hepatic cirrhosis in rats only at the highest dose given (approximately, 20,000 mg/kg body weight/day) (Nilson and Wagner, 1959). Other long-term studies showed effects of weight loss, soft stool, and hepatic changes in rats depending on the source of the carrageenan (Coulston *et al.*, 1975). Besides some randomly-distributed weight loss and chronic intestinal disorders, rhesus monkeys administered carrageenan in the diet for 7.5 years, at doses of 0, 50, 200, or 500 mg/kg body weight, showed dose-related decreases in stool consistency and fecal occult blood over the course of the study, but no gross or microscopic changes in most organs (Abraham *et al.*, 1983). Effects such as the ulcerative lesions seen in guinea pigs and soft stools in the rat and primate studies were most likely due to the physical action of large molecular weight carrageenan in the gastrointestinal tract (JECFA, 1999). Carrageenan was not carcinogenic in rats and hamsters fed 0.5 to 5% (approximately, 250 to 2,500 and 600 to 6,000 mg/kg body weight/day, for rats and hamsters, respectively) in the diet for life (Rustia *et al.*, 1980). Other data suggest that carrageenan may be tumorigenic in the colon of rats when given orally at very high doses (2,100 mg/kg) (RTECS, 1999).

Carrageenan was reported to cause a dose-related increase in colonic cell proliferation in rats but results were only significantly different from controls at the highest doses of 2.6% (1,300 mg/kg body weight/day) (Calvert and Satchithanandam, 1992) and 5% (2,500 mg/kg body weight/day) (Wilcox *et al.*, 1992). In co-carcinogenicity studies, carrageenan at up to 15% in the diet of rats (approximately, 7,500 mg/kg body weight/day), or at 0.25% in drinking-water or 2.5% as a gel (approximately, 250 to 1,250 mg/kg body weight/day) increased numbers of colon tumors induced by *N*-nitrosomethylurea, azoxymethane, and 1,2-dimethylhydrazine (Corpet *et al.*, 1997; Millet *et al.*, 1997; Watanabe *et al.*, 1978; Arakawa *et al.*, 1988). In a single dose study, in which carrageenan was provided to 8 male Fisher 344 rats at 5% (approximately, 2,500 mg/kg body weight/day) in the diet, significantly increased thymidine kinase activity was reported (Calvert and Reicks, 1988).

At JECFA's request for additional data in 1999, a classical initiation-promotion study was conducted in 18 male Fisher 344/DuCrj rats in order to investigate the potential promotion of colon carcinogenesis (Hagiwara *et al.*, 2001). For a period of 4 weeks prior to the commencement of treatment with dietary carrageenan, rats received weekly subcutaneous injections of 20 mg dimethylhydrazine/kg body weight to initiate colorectal carcinogenesis. Subsequently, diets containing *lambda*-carrageenan at concentrations of 0, 1.2, 2.5, or 5.0% (approximately, 0, 600, 1,250, and 2,500 mg/kg body weight/day, respectively) were provided for a period of 32 weeks. In rats receiving 5% carrageenan, with or without initiation, a 5 to 10% reduction in spleen weight was noted compared to the controls. No statistically significant variations in the incidence of colon nodules or tumors were reported between groups of rats initiated with dimethylhydrazine, and there was no significant difference in the incidence of inflammatory lesions. The nodules observed in rats maintained on 5% carrageenan were noted to occur closer to the caecum, compared to all other groups. Under the conditions of this study it was concluded by the authors that carrageenan administered to rats at concentrations of up to 5% in the diet did not promote colorectal carcinogenesis. JECFA did note, however, that previous studies demonstrating promotion of carcinogenesis were conducted at higher doses, and that carrageenan was provided before, during and following initiation with the carcinogen.

Taché *et al.* (2000) investigated the role of the intestinal microflora in the promotion of tumors of the colon in human-floral associated Fisher 344 rats. Administration of carrageenan at 0 or 2.5% in either liquid or as a solid gel was reported to have no effect on the incidence or size of aberrant crypt foci in the colons of human-floral associated rats, and consequently, it was suggested by the authors that the gut flora of rats, but not humans, is associated with the enhancement of colon tumors by carrageenan.

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The effect of carrageenan on the immune system is unclear. In early immunological studies, using subcutaneous, intratracheal, or intralobular routes of administration, carrageenan was reported to induce inflammatory responses in rats and guinea pigs (Robertson and Schwartz, 1953; Di Rosa, 1972; Bowers *et al.*, 1980), and to alter clotting factors (RTECS, 1999). More recent studies suggest carrageenan may have an immunosuppressive effect (Bash and Vago, 1980; Nicklin and Miller, 1984; Nicklin *et al.*, 1988). In ocular and dermal irritation studies, carrageenan was found not to be an irritant to eyes of rabbits, or skin of guinea pigs (Weiner, 1991).
Carrageenan had little to no effects indicating reproductive or developmental toxicity in numerous studies, including multi-generational studies. No evidence of teratogenicity was observed, and decreases in fetal birth weights and slowed skeletal maturation were observed only at very high doses in pregnant mice (up to 900 mg/kg body weight), rats, hamsters and rabbits (up to 600 mg/kg body weight) (Food and Drug Research Labs, Inc., 1972, 1973; Collins *et al.*, 1977a,b, 1979; Vorhees *et al.*, 1979). Carrageenan when injected intravenously into developing chick embryos did induce teratogenic and lethal effects (Rovasio and Monis, 1980); however, the chick is not considered to be an appropriate model for assessing reproductive effects in humans. Additionally, the intravenous route of administration used in the chick study is not relevant to oral exposures.

Genotoxicity/Mutagenicity Data

In vitro Ames tests have shown that *kappa*- and *lambda*-carrageenan were non-mutagenic in strains of *Salmonella typhimurium* and *Saccharomyces cerevisiae* (Brusick *et al.*, 1975). In addition, *kappa*- and *lambda*-carrageenan were not cytogenetic in a host-mediated assay, nor did they cause dominant lethal mutations in rats (Stanford Research Institute, 1972).

Conclusions

The use of carrageenan in food is considered acceptable by the FDA (2002). Furthermore, JECFA (2001) established an ADI of "not specified" for carrageenan, as part of a group ADI that also includes the processed form of the *Eucheuma* seaweed (JECFA, 2001). Although specific exposure levels need to

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be taken into account in an assessment of the safety data, it is not expected that the carrageen in NPcaps™ would raise a safety concern.

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Potassium Chloride (KCL)

Potassium chloride (KCl) is an odorless, colorless crystal, or white crystalline powder, which is prepared from source minerals by fractional crystallization or flotation (Reynolds, 1989; FDA, 2002). It is soluble in water and glycerol, and practically insoluble in alcohol (Lewis, 1989; Reynolds, 1989; FDA, 2002). Potassium chloride is used as an electrolyte replenisher, salt substitute, dietary supplement, flavor enhancer, flavoring agent, nutrient, pH control and gelling agent, and as a stabilizer, thickener, or emulsifier (Lewis, 1989; FDA, 2002). The U.S. Food and Drug Administration (FDA) has affirmed the use of potassium chloride in foods to be Generally Recognized as Safe (GRAS) when used in accordance with Good Manufacturing Practice (GMP) (FDA, 2002). Potassium chloride has been allocated an acceptable daily intake (ADI) of "not limited[2]" by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), as part of a group ADI that includes hydrochloric acid and bases (JECFA, 1967, 1980).

[2] The JECFA designation for ADI of "not limited" refers to their opinion that there is "no need to limit on toxicological grounds the use of hydrochloric acid in accordance with good manufacturing practice" (JECFA, 1967).

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Metabolism Studies

Potassium chloride is readily absorbed from the gastrointestinal tract (Reynolds, 1989). Upon absorption, potassium chloride is expected to be hydrolyzed to its constituent ions, potassium and chloride, which subsequently become participants of normal animal and human metabolic processes (JECFA, 1967). Potassium is excreted primarily in the urine; however, small amounts of potassium have also been identified in the feces, saliva, sweat, bile, and pancreatic juice (Reynolds, 1989). Both potassium and chloride ions are critical in the maintenance of normal acid-base balance, and their excretion and levels in the body are tightly regulated by the kidneys (Saxena, 1989).

Results from a study involving 24 healthy male subjects indicated no significant difference between wax-matrix and liquid formulations of potassium chloride in terms of total amount of potassium excreted in the urine over a 24-hour period (Skoutakis *et al.*, 1984). The absorption and total urinary excretion of potassium after administration of 40 mEq doses of either preparation of potassium chloride were equivalent to approximately 70 to 90% of the administered dose.

Human Safety Data

Potassium chloride, which is available in various oral formulations (*i.e.*, syrups, tablets, and capsules), is the compound of choice for use in the prevention and treatment of hypokalemia with associated hypochloremic alkalosis, since the latter condition can be corrected by the chloride ions (Reynolds, 1989).

Overdose of potassium is not as frequently encountered in clinical practice as hyperkalemia (excess potassium in the body), particularly in patients with compromised renal function (Saxena, 1989). Symptoms of acute potassium toxicity may include cardiovascular changes with ECG abnormalities, neuromuscular effects in the form of general muscular weakness and ascending paralysis, and gastrointestinal symptoms, such as nausea, vomiting, paralytic ileus, and local mucosal necrosis, leading to perforation (Keith *et al.*, 1942; Saxena, 1989).

According to studies reported by BIBRA International Ltd. (1989), no overt adverse effects were noted in groups of 43 normotensive and 32 hypertensive females provided with 108 and 88 mg KCl/kg body weight/day, respectively, for periods of 4 to 6 weeks (Barden *et al.*, 1986; Matlou *et al.*, 1986).

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Volunteers receiving 30 to 124 mg KCl/kg body weight/day in either a wax, microencapsulated, or liquid formulation for a period of 7 days experienced abdominal pains, heart burn, nausea and vomiting (McMahon *et al.*, 1982; Patterson *et al.*, 1983; Ryan *et al.*, 1984; Hutcheon *et al.*, 1988). Upon simultaneous administration of glycopyrrolate (a chemical which delays stomach emptying), it was reported that lesions of the stomach, esophagus, and duodenum were noted in the subjects, with the exception of those provided with the liquid formulation of potassium chloride (McMahon *et al.*, 1982; Patterson *et al.*, 1983; Ryan *et al.*, 1984; Hutcheon *et al.*, 1988). Hypertensive volunteers receiving about 20 mg KCl/kg body weight/day in a microencapsulated formulation experienced constipation, gastric burning and a salty taste in the mouth (Costa *et al.*, 1983), while those provided with 149 mg KCl/kg body weight/day developed abdominal pain and gas; however, blood test results were reported to be within the normal range (Svetkey *et al.*, 1987). In a review of clinical studies conducted by Skoutakis *et al.* (1984), it was concluded that no conclusive evidence exists to suggest that the different slow-release preparations of potassium chloride pose any safety concerns on the upper gastrointestinal tract.
The Boston Collaborative Drug Surveillance Program studied the rates of allergic cutaneous reactions to potassium chloride in patients hospitalized between 1975 and 1982, and reported only one case of positive allergic skin reaction out of 3,460 individuals exposed (Bigby *et al.*, 1986).

Nonclinical Safety Data

In mice, rats, and guinea pigs, oral LD_{50} values of 383, 2,600, and 2,500 mg/kg body weight, respectively, have been determined for potassium chloride (Lewis and Tatken, 1979; Saxena, 1989). Additionally, intravenous LD_{50} values of 117 and 39 mg/kg have been reported for potassium chloride in mice and rats, respectively (Saxena, 1989).
Potassium chloride, administered in an enteric-coated formulation, produced no adverse effects in monkeys dosed with 40 mg/kg body weight/day, and dogs exposed to doses of up to 400 mg/kg body weight/day (Diener *et al.*, 1965). Conversely, groups of rats (gender and strain not specified) administered 5,250 mg KCl/kg body weight/day in the drinking water for a period of 15 weeks

exhibited decreased heart weights, increased kidney weights, and enlarged adrenals. All of these effects were reported to be reversible (Bacchus, 1951). Dietary levels of 0, 1.2, or 3.7% potassium chloride (approximately 0, 850, and 1,850 mg/kg body weight/day, respectively) were administered to groups of weanling Wistar rats (20/sex/group) for a period of 90 days (Mayer *et al.*, 1978). Significantly depressed body weight gains were noted in all potassium chloride-treated males throughout the study period; however, a significant difference in this parameter was noted in females only up to day 29 of the study. No significant differences in cecal weights (empty or filled) or water content of the cecum were observed between potassium chloride-treated and control rats. In a 2-year carcinogenicity study conducted in male F344/Slc rats, no compound-related toxicologically significant effects on parameters of histopathology or tumor incidence were reported in any of the animals exposed to dietary levels of 0.25, 1, or 4% potassium chloride (approximately 125, 500, and 2,000 mg/kg body weight/day, respectively) (Imai *et al.*, 1986).

Genotoxicity/Mutagenicity Data

Potassium chloride produced negative results in the unscheduled DNA synthesis (UDS) assay in HeLa S3 cells, and the Ames assay using *Salmonella typhimurium* strains TA97 and TA102, with or without metabolic activation (Ishidate *et al.*, 1988; Seeberg *et al.*, 1988; Fujita *et al.*, 1992). Conflicting results have been reported in *in vitro* mutagenicity studies in mammalian cells, including the mouse lymphoma L5178Y $TK^{+}/^{-}$ assay and the chromosome aberrations assay. In the absence of metabolic activation, potassium chloride was reported to produce positive (Brusick, 1986) and negative (Mitchell *et al.*, 1988; Myhr and Caspary, 1988) results in the L5178Y $TK^{+}/^{-}$ mouse lymphoma cell forward mutation assay. Similarly, in the chromosome aberrations assay in Chinese hamster ovary (CHO) cells, negative (Ishidate *et al.*, 1988) and positive (Galloway *et al.*, 1985; Seeberg *et al.*, 1988) results were obtained for potassium chloride without metabolic activation. In the presence of metabolic activation, potassium chloride tested positive in the mouse lymphoma cell forward mutation assay, mutation resistance assay in Chinese hamster V79 cells, and chromosome aberrations assay in CHO cells (Galloway *et al.*, 1985; Brusick, 1986; Mitchell *et al.*, 1988; Myhr and Caspary, 1988; Seeberg *et al.*, 1988). Additionally, treatment with 2 M potassium chloride significantly reduced the

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survival, and enhanced the mutation frequency of logarithmic-phase, but not stationary-phase cells, of *Saccharomyces cerevisiae* strain XV185-14C (Parker and von Borstel, 1987).
Notwithstanding the conflicting results of *in vitro* assays, a 2-year carcinogenicity study in rats showed no evidence of carcinogenicity following the administration of potassium chloride *in vivo* (Imai *et al.*, 1986).

Conclusion

The use of potassium chloride in food is considered acceptable by the FDA (2002). Furthermore, JECFA (1967; 1980) established an ADI of "not limited" for potassium chloride, as part of a group ADI that includes hydrochloric acid and bases. Although specific exposure levels need to be taken into account in an assessment of safety data, it is not expected that the potassium chloride in NPcaps™ would raise a safety concern.

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Regulatory Status of Final Formulation for Use as a Dietary Supplement

NPcaps™					
DS Market Application	**Japan**	**United States**	**Canada**	**Australia**	**EU**
Pullulan	Yes	Yes	Yes[1,2]	Yes[1]	Yes[1]
Carrageenan	Yes	Yes	Yes	Yes	Yes
Potassium Chloride	Yes	Yes	Yes	Yes	Yes
Composite	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**

[1] Not listed as a food additive in this jurisdiction. Adequate safety data exist to support the use of this ingredient.
[2] Listed for use in marketed cosmetic and personal care products.

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Regulatory Status of Final Formulation for Use as a Drug

NPcaps™					
Pharmaceutical Market Application	**Japan**	**United States**	**Canada**	**Australia**	**EU**
Pullulan	Yes[1]	Yes[1]	Yes[1,3]	Yes[2]	Yes[2]
Carrageenan	Yes[1]	Yes	Yes	Yes	Yes
Potassium Chloride	Yes	Yes	Yes	Yes	Yes
Composite	**Yes[4]**	**Yes[4]**	**Yes[4]**	**Yes[4]**	**Yes[4]**

[1] No relevant pharmacopoeial standard identified. Refer to use as food additives. Inactive ingredients that are generally recognized as safe for use in foods typically can be used in pharmaceutical drug products. These ingredients typically need to conform to a manufacturer's Certificate of Analysis.
[2] No relevant pharmacopoeial standard identified. Not listed as a food additive in this jurisdiction. Adequate safety data exist to support the use of this ingredient.
[3] Listed for use in marketed cosmetic and personal care products.
[4] Qualitatively, the formulation would be suitable for use based on its inactive ingredients. A manufacturer's Certificate of Analysis would be required. Additional testing may be necessary depending on the active ingredient.

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Pullulan

What are pullulan capsules?

- Capsules composed of an edible material derived from corn syrup

What is this material?

- Its formal chemical name is pullulan (not a brand name...just like "gelatin" is not a brand)
- It is a carbohydrate, and is considered a "simple polysaccharide"
- Textbook definition of pullulan:
  - "A water-soluble polysaccharide composed of glucose units that are polymerized in a way as to make it viscous and impermeable to oxygen."

What does pullulan look like?

- Tasteless, odorless white powder
- Solutions of pullulan can dry to films
- Films are quickly soluble in water, have low oxygen permeability and are glossy

How long has it been around?

- First reported in 1938
- First commercial production in 1976, as a food additive (e.g. thickener); consumed safely in Japan for over 20 years
- Most recognized US use of pullulan is the film for Listerine Pocketpaks™

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How is pullulan made?

- Uses a fermentation process, where a yeast-like organism (*aureobasidium pullulan*) acts on a food source (*corn syrup; certified GMO-free*)
- From Corn to Pullulan:
  - Corn starch comes from "starchy" (chains of glucose) part of corn. Starch is how plants store energy (glucose)
  - To make corn syrup, enzymes are added to corn starch, to turn it into a syrupy mixture of simple sugars (e.g. glucose, dextrose, maltose)
  - *A. pullulans* acts on corn syrup, stringing the sugars together into a simple polysaccharide called pullulan
  - In your body, pullulan is digested like many other common carbohydrates

Pullulan Production Overview

- Conducted under GMP conditions; raw materials meet food grade specifications
- Fermentation of corn syrup with *A. pullulans*
- Micro-filtration to remove *A. pullulans*
- Cell-free filtrate is heat sterilized; absence of *A. pullulans* is confirmed
- Filtrate is decolorized, filtered and de-ionized; then evaporated; then filtered again
- Dried and pulverized to form granules

More About A. Pullulans

- Abundant in nature (decaying trees, exterior windows, breweries, paper mills, etc.)
- Non-toxic
- Non-pathogenic
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- Human consumption study showed no symptoms; clear evidence that colonic activity completely digests pullulan
- Long-term rat studies show no negative effects

Regulatory Status

- US
  - GRAS (Generally Recognized as Safe)
    - Granted on Aug. 1, 2002 by FDA
    - GRAS Notice #: GRN 0000999
- Japan
  - Approved as food ingredient and additive; no limitation of use
    - Serial #373; April 16, 1996
- Europe
  - Under review as Food Additive

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